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Levels of a dominant Glutathione *S*-transferase in onion bulbs have a seasonal relationship with physiological inhibitors

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Abstract

Isoforms of onion bulb GSTs (designated as GSTa, GSTb, as minor, and GSTc, GSTd and GSTe as dominant GSTs) were separated by DEAE-cellulose chromatography, and variation in the activity of dominant GSTs was studied in winter and spring. Among the dominant GSTs, GSTc and GSTd were found to have similar activity levels in both seasons. In case of GSTe, distinct activity levels were observed in winter and spring, where, a decreased activity was found in spring. An expressional relation was observed between GSTe and physiological inhibitor, quercetin-4´-glucoside in onion calli. However, GSTe was not responsive to quercetin-3,4´-diglucoside. The concentrations of the two inhibitors were also compared in onion bulb tissues collected from winter and spring seasons. Quercetin-4´-glucoside and quercetin-3,4´-diglucoside containing fractions, ethylacetate (EtOAc) and water fraction, were isolated from onion bulb extract, and IC_{50}s of the fractions were measured on GSTc, a sensitive GST to the inhibitors. The IC_{50}s of EtOAc- and water fraction on GSTc (in winter 84 and 283 mg ml^{-1} fresh tissue, respectively, and in spring 102 and 214 mg ml^{-1} fresh tissues, respectively) and HPLC profile suggested that the concentration of quercetin-4´-glucoside decreased over time of storage in onion bulb, while the concentration of quercetin-3,4´-diglucoside increased in spring. Therefore, lower activity of GSTe in onion bulb in spring is due to lower content of quercetin-4´-glucoside in onion bulb tissues, and hence, quercetin-4´-glucoside might have important role in expressing the activity of this GST in onion bulb.

Keywords: Onion GSTs; Seasonal change; Relation; Physiological inhibitor; Quercetin-4´-glucoside

Abbreviations

BSA_Bovine serum albumin; CDNB_1-Chloro-2,4-dinitrobenzene; DEAE_ Diethylaminoethyl; DW_Distilled water; EDTA_Ethylene diamine tetraacetic acid; EtOAc_Ethylacetate; GST_Glutathione *S*-transferase; HPLC_High-performance liquid chromatography; IAA_Indole-3-acetic acid; IC_{50}_50% Inhibition concentration; i.d._Internal diameter; NAA_Naphthelinacetic acid; SA_Salicylic acid; SE_Standard error; TLC_Thin layer chromatography; 2,4-D_2,4-dichlorophenoxy acetic acid; 2,3-D_2,3-dichlorophenoxy acetic acid; 2,3,6-T_2,3,6-trichlorophenoxy acetic acid

Introduction

Glutathione *S*-transferases (GSTs, E.C.2.5.1.18) have been associated with detoxification of xenobiotics, limiting oxidative damage and other stress responses in plants (Gong et al., 2005). The well-studied detoxification reactions of GSTs ultimately lead to vacuolar sequestration of substrates conjugated to glutathione (GSH). As non-enzymatic carrier proteins (ligandins) GSTs enable intracellular shuttling of endogenous compounds in living organism. However, information about the function of GSTs with endogenous cytotoxic compounds is still limited. Three GSTs including maize BZ2 (Marrs et al., 1995), petunia AN9 (Alfenito et al., 1998; Mueller et al., 2000) and Arabidopsis TT19 (Kitamura et al., 2004) have been identified and characterized for their involvement in transportation of endogenous substrate, anthocyanin into vacuoles. GSTs have also been shown to bind other flavonoids *in vitro* and to be important for their transport (Mueller et al., 2000; Winefield et al., 2006). A Recent study characterized some flavonoid binding proteins, and demonstrated strong correlation between GST proteins and anthocyanin accumulation in grape (Conn et al., 2008). Therefore, like anthocyanin other endogenous substrates of GSTs might have important relation with

the expression level of GST activity. However, information in this regards have been limited.

Previously, the GSTs activity in onion bulb has been enriched when compared to other vegetable crops (Hossain et al., 2007). Therefore, onion bulbs and calli have been studied for GSTs and their endogenous substances. Onion bulb GSTs consist of five component GSTs including GSTa, GSTb, GSTc, GSTd and GSTe (Rohman et al., 2009a). Among those, GSTa and GSTb, with low activity, were termed as minor GSTs, and GSTc, GSTd and GSTe, with high activity, were termed as dominant GSTs. In onion bulb, quercetin-4´-glucoside and quercetin-3,4´-diglucoside were also reported as physiological inhibitors of the dominant GSTs (Rohman et al., 2009a,b). The 1-Chloro-2,4- dinitrobenzene (CDNB) conjugating activities of GSTc and GSTd, and to a lesser extent GSTe, were highly sensitive to the inhibitions of the inhibitors, particularly quercetin-4´-glucoside. Recently, we observed that the activity level of the dominant GST, GSTe was found to be changed over time of storage of onion bulb. Therefore, this study was conducted to examine the variation in the activities of the dominant GSTs in onion bulb collected from winter (December) and spring (May) seasons. The most studied endogenous substrate, anthocyanin that has been reported to have a positive correlation with GST accumulation, is also a strong inhibitor of the GSTs (Mueller et al., 2000; Cummins et al., 2003). Therefore, endogenous inhibitory substrates of onion GSTs might also affect the activity levels of the GSTs in onion bulb. In this study, the variation in the activities of dominant onion bulb GSTs in winter and spring season were described. The expressional relations between dominant GSTs with their endogenous inhibitors, quercetin-4´-glucoside and quercetin-3,4´-diglucoside, were also studied.

Materials and methods

Plant materials

Onion bulbs (*Allium cepa* L.), variety "Senshyu Yellow" were collected from local market and preserved at 4ºC. Before experimentation, onion bulbs were exposed to 25ºC for 24 h and the activity levels of GSTs and the concentrations of their inhibitors were examined in winter (December) and spring (May). Callus was induced from onion seed (same variety) cultured in liquid Murashige and Skoog (MS) liquid medium (Murashige and Skoog, 1962) containing 4.5 µM 2,4-D and 0.5 µM kinetin at 25ºC. After 3 subcultures, propagated calli were subjected to treat with 200 µM of quercetin-4´-glucoside and quercetin-3,4´-diglucoside in liquid MS media, and shaken for 72 hours at 25ºC. Callus transferred on MS liquid medium maintained in the same condition was used as control.

Separation of isoforms of GSTs from onion bulb and calli

Crude enzymes were extracted by homogenizing of fresh onion bulb tissues and calli in an equal volume of 25 mM Tris-HCl buffer (pH 8.5), which contained 1 mM EDTA and 1% (w/v) ascorbate, with a Waring blender. The homogenates were squeezed through two layers of nylon cloth and centrifuged at 11,500×*g* for 10 min. Proteins were precipitated by ammonium sulfate at 65% saturation from the supernatant, and centrifuged at 11,500×*g* for 10 min. The proteins were dialyzed against 10 mM Tris-HCl buffer (pH 8) containing 0.01% (w/v) β-mercaptoethanol and 1 mM EDTA (buffer A) overnight to completely remove low molecular inhibitors. The dialyzate was put on a column (1.77 cm i.d. × 20 cm) of DEAE-cellulose (DE-52, Whatman, UK) that had been equilibrated with buffer A and eluted with a linear gradient of 0 to 0.2 M KCl in 600 ml of buffer A. GST activities of the fractions were assayed spectrophotometrically.

Preparation of enzyme from inhibitor treated calli

One gram (g) of calli were homogenized with an equal volume of 25 mM Tris-HCL buffer (pH 8.5) that contained 1 mM EDTA and 1% (w/v) ascorbate (buffer A) using a mortar and pestle. Homogenates were centrifuged at 11,500×*g* for 10 min, and then the supernatants were used as enzyme solution.

Assay of enzyme activities and protein quantitation

GST activity was determined spectrophotometrically by the method of Booth et al. (1961) with some modifications. The reaction mixture contained 100 mM potassium phosphate buffer (pH 6.5), 1.5 mM GSH, 1 mM CDNB, and enzyme solution in a final volume of 0.7 ml (with and without inhibitors). The enzyme reaction was initiated by the addition of CDNB, and A_{340} was measured at 25ºC for 1 min. The activity was expressed as nmol min^{-1} mg^{-1} protein using the extinction coefficient of CDNB, ε_{340} = 9.6 mM^{-1} cm^{-1}. The protein concentration of each sample was determined by the method of Bradford (1976) using BSA as protein standard.

Production of polyclonal antibody against GSTe

GSTe was purified by DEAE-cellulose, hydroxyapatite and affinity column chromatography following the method of Fujita and Hossain (2003). A rabbit received subcutaneous injections of a 0.5 mg of purified GSTe protein in Freund's complete adjuvant at several sites. After two weeks, the rabbit was given a first booster injection of 0.5 mg of the purified GSTe protein in incomplete adjuvant. A second booster injection of 0.5 mg of the purified protein in incomplete adjuvant was

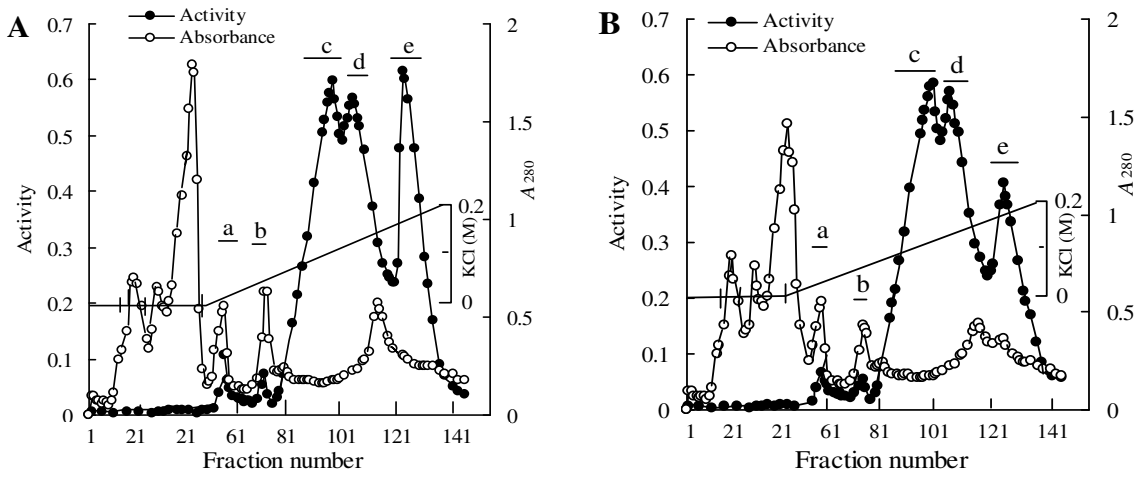

Fig 1. Seasonal variation in the activity of onion bulb GSTs in winter (A) and spring (B). The GSTs were separated by DEAE-cellulose chromatography. For each fraction absorbance at 280 nm (○) and activity towards CDNB (●) were determined. The activity was expressed as µmol min^{-1} ml^{-1}. The bars indicate the high active fractions of each GST isoform. The letters show the convenient names of the GSTs: a, GSTa; b, GSTb; c, GSTc; d, GSTd and e, GSTe. Upper line indicates KCl gradient. Fig A is adopted from Rohman et al. (2009b).

given after two weeks of the first booster injection. Blood was taken from the ear vein one week after the second booster injection and centrifuged at 3,400×*g* for 4 min. The antiserum was used as antibody of GSTe. Anti-*Cm*GSTF1 antiserum (Rohman et al., 2009a) was used to recognize the expression of GSTc in soluble protein extracts.

Western blotting

The soluble protein extracts of onion calli treated with endogenous inhibitors were subjected to Western blot analysis: SDS-PAGE (12.5% gel) according to Laemmli (1970). After electrophoresis, polypeptides were electroblotted onto nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Biotech, UK) and probed with antibody of GSTc and GSTe, followed by a secondary anti-rabbit immunoglobulin (IgG) goat antibody coupled with horseradish peroxidase. The antibody-antigen complexes were then visualized by an enhanced chemiluminescence assay as detailed by the manufacturer (PerkinElmer Life Sciences, Inc.).

Preparation of quercetin-4´-glucoside and quercetin-3,4´-diglucoside containing fractions from onion bulb

Quercetin-4´-glucoside and quercetin-3,4´-diglucoside containing fractions were prepared from mature onion bulb of winter and spring as described by Rohman et al. (2009a,b) briefly; 20 g of fresh material was extracted three times with 50% methanol. The extracts were evaporated and suspended in 15 ml distilled water (DW) and divided into three fractions; *n*-hexane soluble fraction (*n*-hexane fraction), ethylacetate (EtOAc) soluble fraction (EtOAc faction), and water fraction (remaining extract). EtOAc fraction (0.5 ml equivalent to 20 g fresh onion bulb tissue) were applied into a solid phase extraction kit (Sep-pak Vac C_{18} column, Waters, Ireland) and eluted with 20 ml of 30% methanol followed by the same volume of 45, 65 and100% methanol. The water fraction was eluted with same amount of 0, 20, 45, 65 and 100% methanol. The fractions containing quercetin-4´-glucoside and quercetin-3,4´-diglucoside were prepared form 45% methanol elutes of EtOAc- and water fraction, respectively.

High performance liquid chromatography (HPLC)

HPLC analysis was carried out on an LC-6AD Liquid Chromatograph (Shimadzu, Japan) fitted with a UV-VIS detector (SPD-6AV) and C-R6A Chromatopac. Separation was performed onto an Inertsil ODS-3 column (4.6 mm i.d. × 100 mm, GL Sciences Inc., Japan) with a methanol gradient from 30 to 80% for 40 min and at 100% for 20 min. The flow rate was 0.6 ml min^{-1} and detection was carried out at 220 nm.

Results and discussion

The component GSTs (GSTa, GSTb, GSTc, GSTd and GSTe) were separated from crude enzyme by DEAE-cellulose chromatography in winter and spring, and variation was observed in the activities of dominant GSTs, GSTc, GSTd and GSTe. The activity towards CDNB and absorbance (A_{280}) of the DEAE fractions are shown in Fig 1. In both season, five peaks of GST activities (GSTa, GSTb, GSTc, GSTd and GSTe) eluted at approximately 43, 65, 106, 117 and 157 mM KCl. The activities of GSTc and GSTd were similar in both seasons, however, a different phenomenon was observed for GSTe. Higher GSTe activity was observed in winter and the activity decreased in spring.

The activity of GSTe showing variation with distinct

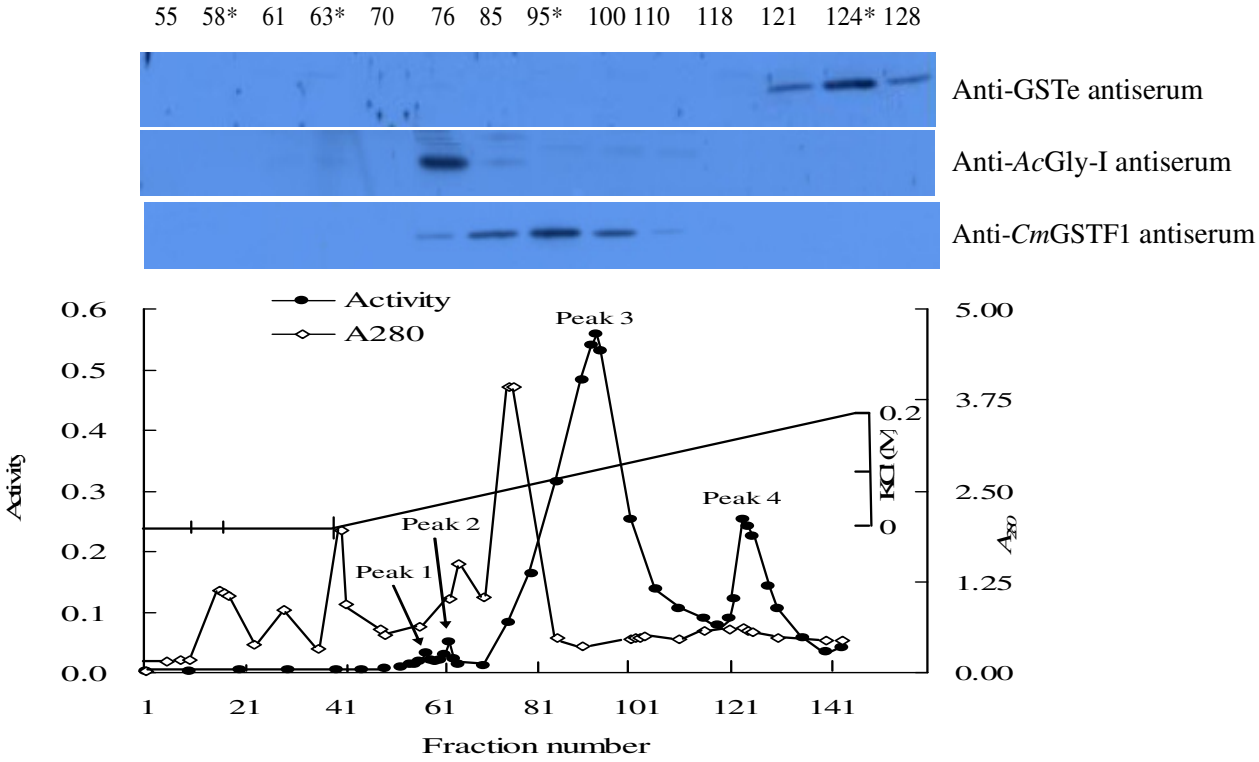

Fig 2. A typical column chromatography of DEAE-cellulose of soluble proteins prepared from 80 g onion calli. For each fraction, absorbance A_{280} nm (○) and GST activity toward CDNB (●) were determined. Activity is expressed as µmol $min^{-1}ml^{-1}$. The upper figure shows the results of Western blot analysis of selected fractions by the antibodies of GSTc, GSTe and by anti-*Ac*Gly-I antiserum. * indicates the peak fractions.

high in winter and low in spring might have biological significant. Higher activities of several GSTs in low temperature have been reported showing important role in winter hardening (Anderson and Davis, 2004). Therefore, higher activity of GSTe in winter might involve in cold tolerance. On the other hand, GSTs have been reported to have strong transcriptional relationship with their endogenous substrate like anthocyanin (Conn et al., 2008). In previous experiments, high interactions of dominant onion bulb GSTs with physiological inhibitors were observed, where the CDNB-conjugating activities of GSTc and GSTd were highly sensitive to inhibition of quercetin-4´-glucoside and quercetin-3,4´-diglucoside, particularly to quercetin-4´- glucoside. GSTe had comparatively lower sensitivity to the inhibition (Rohman et al., 2009a,b). The endogenous GST substrate, anthocyanin, reported to have transcriptional relation with GSTs, is also an inhibitor of GSTs (Mueller et al., 2000; Cummins et al., 2003). Therefore, endogenous inhibitory substrates might have expressional relation with the activity of GSTe and that might affect the activity of the GST. To test the possibilities, antibody of GSTe was produced in rabbit serum against the purified GSTe. GSTe was purified from onion bulb using a combination of ammonium sulfate fraction, ion exchange, hydroxyapatite and *S*-hexylglutathione-agarose column chromatography. In cross reactivities of five GST isoforms of onion bulb, the antiserum detected only GSTe (data not shown) and therefore, this antiserum was used as antibody of GSTe. Anti-*Cm*GSTF1 antiserum (Rohman et al., 2009a) was used to detect the expression of GSTc. The expression levels of GSTe as well as GSTc were tested in onion calli treated with 200µM quercetin-4´-glucoside and quercetin-3,4´-diglucoside in liquid MS medium containing 4.5 µM 2,4-D and 0.5 µM kinetin at 25ºC for 72 h. A control treatment was maintained under the same condition. The culture tubes were shaken on a rotor to avoid the oxidative stress. However, since the expressions of GSTc and GSTe are being tested in onion calli, question may arise, whether these isoforms are present in onion calli. To remove this perplexity, the callus GSTs were separated by DEAE-cellulose chromatography, and cross reactivity of selected GST fractions was tested by antibodies of GSTc and GSTe (Fig 2). The cross reactivity of the fractions was also test by anti-*Ac*Gly-I antiserum, because onion bulb also shows high Glyoxalase-I activity (Hossain et al., 2007).

Onion calli have four GST peaks eluted at approximately 40, 60, 106 and 157 mM KCl (Fig 2). The cross reactivities of the selected high active GST fractions showed that antibody of GSTc reacted only with the GST fractions of peak 3, while antibody of GSTe reacted only with the GST fractions of peak 4. Anti-*Ac*Gly-I antiserum showed cross reactivity with none of the fractions of peak 3 and peak 4. Since peak 3 and peak 4 eluted at same fraction numbers (95 and 124) and at the same KCl gradient (106 and 157 mM, respectively) like GSTc and GSTe from onion bulb, they might represent GSTc and GSTe, respectively. All GST isoforms of a particular organ might not express in all organs of same plant, for example, *Zm*GST I and *Zm*GST V were expressed more in roots than in shoots,

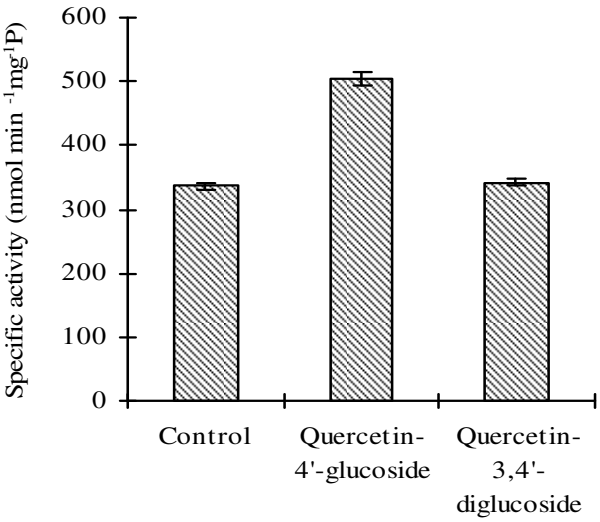

Fig 3. Effects of quercetin-4´-glucoside and quercetin-3,4´-diglucoside on GST activities in soluble extracts of onion calli treated with quercetin-4´-glucoside and quercetin-3,4´-diglucoside at 200µM concentration in MS medium for 72 h. GST activity was assayed towards CDNB. The experiment was repeated three times and bars indicated SE.

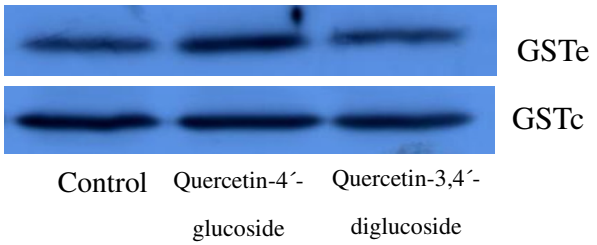

Fig 4. Western blotting showing the expression of GSTe and GSTc in soluble extract of onion calli treated with quercetin-4´-glucoside and quercetin-3,4´- diglucoside at 200µM in MS medium at 25ºC for 72h. Each lane contained 35 µg of the protein.. This experiment was conducted three times and the result is shown from one experiment.

but *Zm*GST II expression was limited in the roots (Dixon et al., 1998). In *Arabidopsis*, one GST homolog, At103-1a, has been shown to express in the green part of the plant but expression of another GST gene, At103-1b, can be detected in both green plant part and root (van der Kop et al., 1996). Results of the present study also showed that expression of onion GST isoforms is organ specific.

GST activity was measured towards model substrate CDNB in soluble extracts prepared from onion calli treated with quercetin-4´-glucoside and quercetin-3,4´-diglucoside (Fig 3). It was observed that the calli treated with quercetin-4´-glucoside induced 1.5 fold GST activity compared to control suggesting inducible relation with GSTs. However, none of the inhibitors affected the activity of GSTc. Therefore, the expression of GSTe was investigated in the soluble extracts by Western blotting to find whether these

Table 1. Inhibitory activities of quercetin-4´-glucoside and quercetin-3,4´-diglucoside containing fractions, EtOAc and water fraction, respectively, on GSTc in winter and spring. Twenty g fresh onion bulb tissue was extracted and divided into *n*-hexane-, EtOAc- and water fraction. Activity of GSTc was taken towards CDNB in function of EtOAc- and water fraction prepared from onion bulb extract. IC_{50}s were graphically determined and expressed as mg ml^{-1} fresh onion bulb tissue, from which extracts were prepared. The data presented in the table were obtained from 3 independent experiments.

Extracts	IC_{50} (mg ml^{-1} fresh tissue)	
	Winter	Spring
EtOAc fraction	84±4.1	102±3.5
Water fraction	283±5.8	214±3.2

endogenous inhibitory substrates have any role in inducing the activity of GSTe. The expression of GSTc, the most dominant GSTs in onion bulb, was also checked, because the induced activity in soluble protein extracts might also be a contribution of GSTc. However, neither quercetin-4´-glucoside nor quercetin-3,4´-diglucoside showed increased accumu- lation of GSTc (Fig 4). The expression of GSTe was found to be induced only in quercetin-4´-glucoside treated calli. The cellular effects of quercetin are complex and include both antioxidant effects and induction of oxidative stress due to formation of reactive oxygen species in the extracellular medium. Thus the amount of glutathione might be increased in cells. Smith et al. (2003) demonstrated that expression of one of the GST isoforms is induced by glutathione in Arabidopsis. A similar way of regulation may exist in onion. The expression of GSTe in the soluble extracts of the calli treated at 4ºC and 25ºC was also exmanied, but low temperature did not affect the expression of GSTe (data not shown). Therefore, induced expression of GSTe in quercetin-4´-glucoside treated calli suggested an expressional relation between them.

As non-enzymatic carrier protein (ligandin) GSTs are reported to transport endogenous compound in maize, *Arabidopsis*, petunia and carnation (Marrs et al., 1995; Alfenito et al., 1998; Mueller et al., 2000; Larsen et al., 2003). However, information on transcriptional or expressional relationship of GSTs with endogenous substrate has been limited. Within plants, ligands include the ubiquitous plant pigments, anthocyanins, IAA, NAA, labile cytochrome precursors (Jones, 1994; Bilang and Sturm, 1995; Marrs et al., 1995; Mueller et al., 2000; Lederer and Böger, 2005). The soybean GH2 /4 gene is inducible not only by strong auxins (2,4-D; 2,4,5-T; α-NAA) and SA, but also by numerous other electrophilic compounds, including weak auxins (β-NAA), inactive auxin analogues (2,3-D and 2,3,6-T), and inactive salicylic acid (SA) analogues (3-hydroxybenzoic acid, 4-hydroxybenzoic acid) (Ulmasov et al., 1994, 1995). Furthermore, the *Hm*gst-1 protein from *Hyoscyamus muticus* able to bind IAA exhibiting an expression profile that correlates with accumulation of IAA, suggesting that

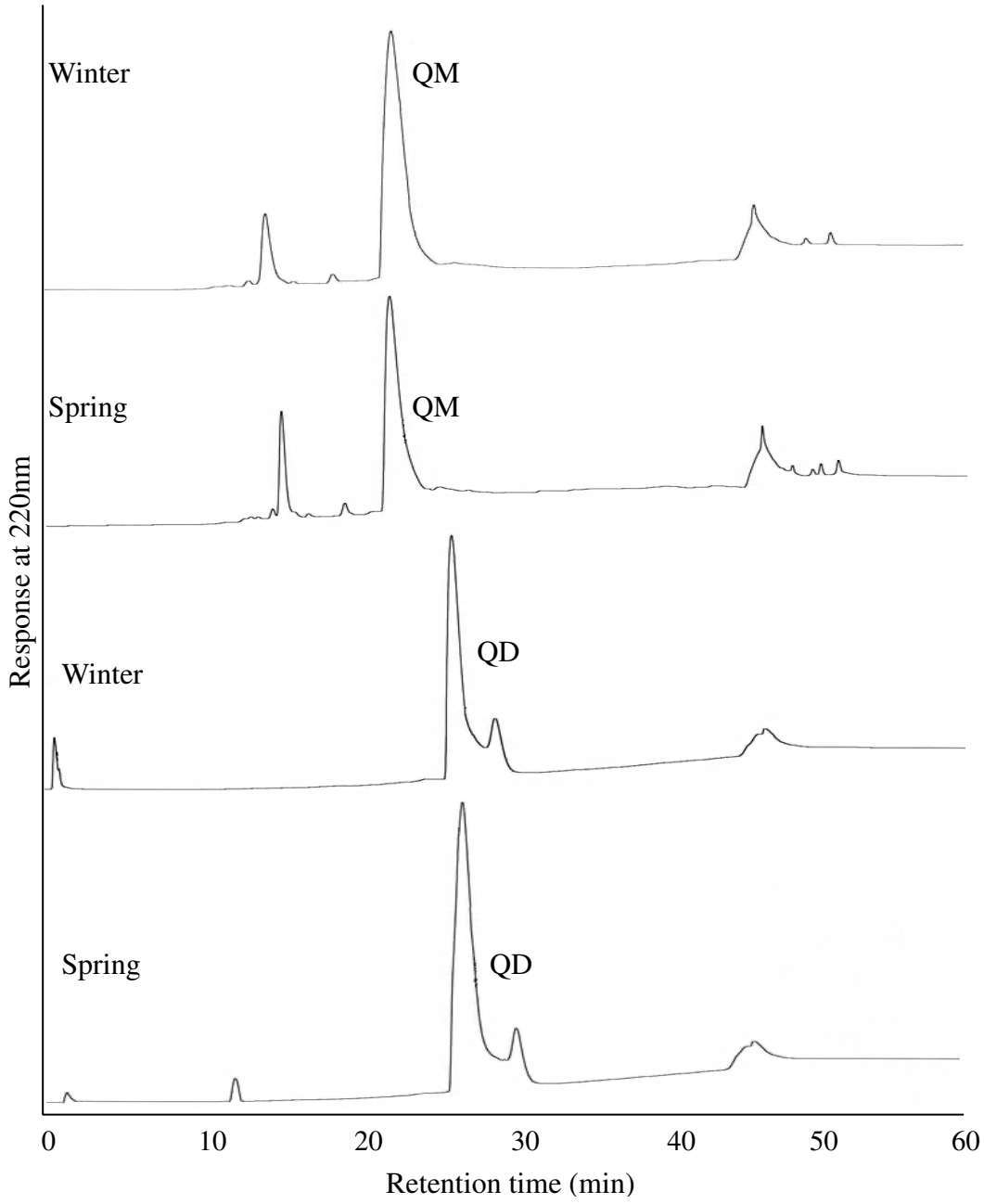

Fig 5. Comparative HPLC profile of quercetin-4´-glucoside and quercetin-3,4´- diglucoside in EtOAc- and water fraction, respectively, in onion bulb in winter and spring. For each case, 5 µl sample equivalent to 150 mg fresh onion bulb tissue for quercetin-4´-glucoside and 600 mg onion bulb fresh tissues for quercetin-3,4´-diglucoside was injected. QM, quercetin-4´-glucoside and QD, quercetin-3,4´- diglucoside.

this GST is involved in intracellular IAA transport (Bilang et al., 1993; Bilang and Sturm, 1995). Beyond this work on auxins, few studies relate GST transcription or expression with accumulation of endogenous substrates. Two putative anthocyanin transport proteins, *Vv*GST1 and *Vv*GST4 in grape have been identified and found a strong correlation with accumulation of endogenous substrate anthocyanin (Conn et al., 2008). The anthocyanin contents are accumulated in grape cell suspension cultures treated with continuous white-light irradiation, sucrose, and jasmonic acid, and the accumulation has a positively correlation with the GST levels. However, the information that endogenous substrate can induce activity in *in vitro* studies has not been reported. The results of this experiment suggested that the concentration of quercetin-4´-glucoside is an important factor in controlling the expression of GSTe in onion bulb. Therefore, the concentration of quercetin- 4´-glucoside was compared in onion bulb in winter and spring.

To compare the concentrations of quercetin-4´-glucoside and quercetin-3,4´-diglucoside, the inhibitory fractions (*n*-hexane-, EtOAc- and water fraction) were extracted from onion bulbs in winter and spring as described in materials and methods. IC_{50}s of quercetin-4´-glucoside and quercetin-3,4´-diglucoside containing fractions, EtOAc fraction and water fraction, respectively, were measured on GSTc and expressed as mg ml^{-1} fresh tissues (Table 1).

The IC_{50}s of EtOAc fraction were obtained from 84

and 102 mg ml^{-1} fresh tissues in winter and spring, respectively and in water fraction, they were 283 and 214 mg ml^{-1} fresh tissues in winter and spring, respectively. Therefore, the IC_{50}s values on GSTc suggested that the contents of inhibitory substances changed over time of storage, where in spring, quercetin-4´-glucoside was seemed to be decreased, and quercetin-3,4´-diglucoside increased. However, for more precious information, the fractions were applied on a solid phase extraction kit (Sep-pak Vac C_{18} column, Waters, Ireland) and eluted with different concentrations of methanol, and inhibitor containing fractions (for both cases 45% methanol-elution) were collected for HPLC analysis. HPLC analysis was done with 5 µl sample equivalent to 150 mg fresh onion bulb tissue for quercetin-4´-glucoside and 600 mg fresh onion bulb tissues for quercetin-3,4´-diglucoside were injected. The HPLC profile of quercetin-4´-glucoside and quercetin-3,4´-diglucoside in EtOAc and water fraction, respectively, are summarized in Figure 5.

The HPLC profiles preciously indicated that concentration of quercetin-4´-glucoside in onion bulb tissue was higher in winter than in spring. On the other hand, concentration of quercetin-3,4´-diglucoside increased in spring. The stability of the major flavonol glucosides, quercetin-4´-glucoside and quercetin-3,4´-glucoside were studied in two varieties of onion (Red Baron and Crossbow) that were stored for 6 months under normal commercial conditions and analyzed at regular intervals (Price et al., 1997). They reported that the concentrations of these two compounds change for subsequent storage period. Changes in content of flavonoid compounds have also been reported in lettuce, red raspberry and endive (DuPont et al., 2000; Zafrilla et al., 2001). The lower concentration of quercetin-4´-glucoside in onion bulb tissue in spring might be due to deglucosidation process through conversion to its aglycon, quercetin (Takahama and Hirota, 2000). Moreover, quercetin-4´-glucoside might be modified to diglucoside, as plants modify self produce toxic metabolites into less toxic form via glycosylation (Sirikantaramas et al., 2007), which might ultimately increase the concentration of quercetin 3,4´-diglucoside in spring.

The results presented in this study demonstrated the variation in te activity of one GST isoform, GSTe in onion bulb over time of storage, and GSTe has strong expressional correlation with the concentration of inhibitory substrate, quercetin-4´-glucoside in onion bulb. Both the activity of GSTe and concentration of quercetin-4´-glucoside were higher in winter and became lower in spring. Therefore, the expressional relationship between them suggested that lower activity of GSTe in onion bulb in spring is due to the lower concentration of the physiological inhibitor, quercetin-4´-glucoside.

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